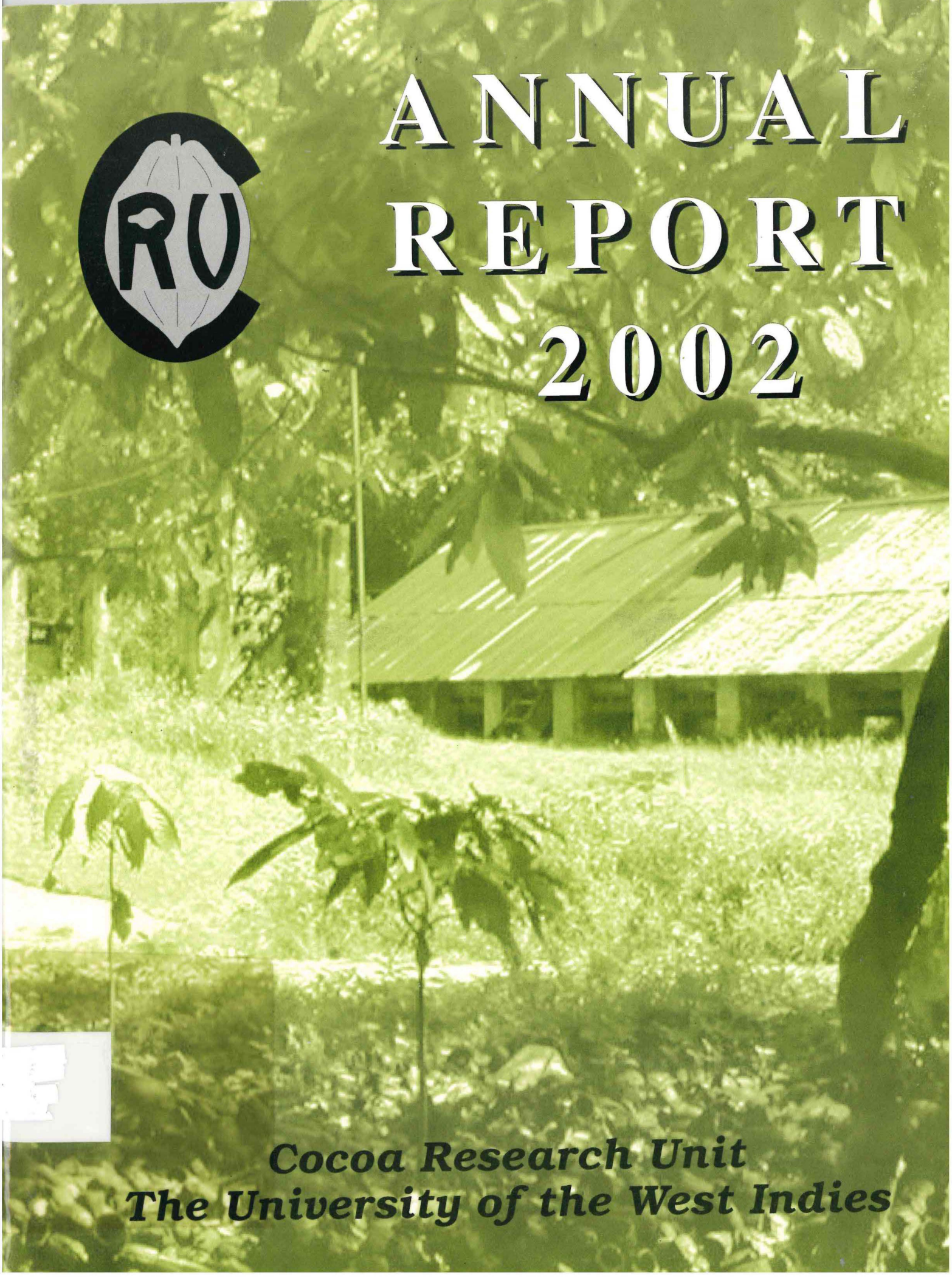
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Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago

Tel. +1 868 662 8788
+1 868 662 4996 Ext. 2115
+1 868 645 3232 2115
Fax +1 868 662 8788
E-mail cru@cablenett.net

Cover photograph. "Cocoa houses" for sun drying of beans in a traditional cocoa estate, north Trinidad.

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Mass screening for resistance to Witches' Broom disease under greenhouse conditions

R. Umaharan, J-M. Thévenin, Y. Mosca and J. Bhola

Introduction

Screening of grafted clones from the ICG,T for resistance to Witches' Broom disease commenced in July 1998. The aim of this programme has been to identify clones in the ICG,T which show a high level of resistance to this disease. The work undertaken has involved the adaptation of an automated spray inoculation system and the standardisation of this method for carrying out mass screening at the CRU (Umaharan *et al.*, 2001), in addition to the inoculation of grafted clones with *Crinipellis perniciosa.* Details of the progress of this work are outlined in this report.

Materials and Method

Inoculation conditions

In all experiments, inoculations were carried out by spraying, employing either a modified version of the automated overhead spray system (Purdy *et al.*, 1997) or manually using a Preval Sprayer (Precision Valve Corp., NY, U.S.A), which delivers a fine spray equivalent to the automated overhead spray system. When spraying manually, inoculation was performed in a similar way to the overhead spray system i.e. inoculum was delivered from the top of the plants down, in a regular and even movement.

The dose rate of inoculum was similar for all experiments, using a concentration of 350,000 basidiospores mL^{-1}, at a rate of 1.0-1.4 mL $plant^{-1}$. After inoculation, plants were kept at 23-25°C, not less than 90% relative humidity and in the dark for 60 hours.

Confirmation of resistance in promising accessions

Additional experiments have been undertaken to implement the agar-droplet method (Surujdeo-Maharaj *et al.*, 2003), for verification/reconfirmation of promising accessions evaluated with the spray method. This technique involves the inoculation of individual shoots using an inoculum suspension in 0.3% agar. The same incubation conditions were applied, as described above.

A comparative experiment was undertaken where 27 accessions were inoculated using both the spray and agar-droplet techniques on the same plant. The plants selected for this experiment had two distinct branches which could be inoculated and assessed separately.

The agar-droplet technique was also used to screen TSH material for MALMR as part of the CFC/ICCO/IPGRI Project.

Clones were assessed based on percentage infection i.e. total number of brooms, swellings and necroses which developed out of the total number of shoots inoculated per clone, calculated as a percentage, and largest broom-base diameter.

Results

Grafting of Clones

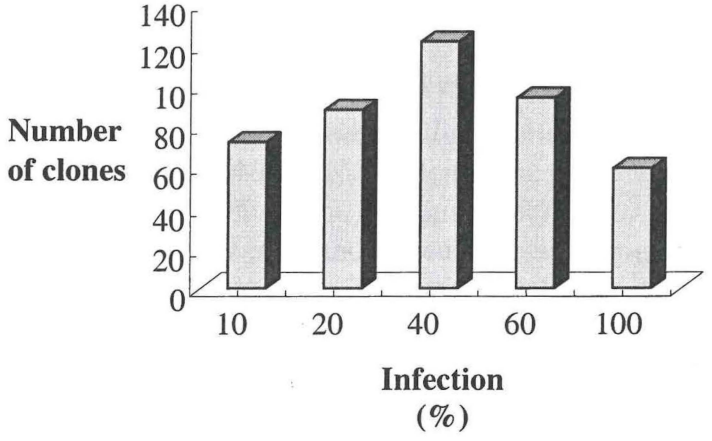
Between January and November 2002, 11 series of plants were grafted at a rate of 50 accessions per series, and 15 replicates per accession (giving 750 plants per series). We have completed an additional 550 accessions this year, bringing the total number of accessions grafted since the start of the project to 1,065. To achieve this, over 22,200 rootstocks have been established and grafted since the start of the project.

Screening

During 2002, a total of 385 accessions were inoculated, representing eleven series each with an average of 200 plants. At least 35 accessions were screened in each series. Of the 385 accessions, 160 are currently being observed for symptoms. Since the start of the project, 570 clones have been inoculated (in 19 series), representing 47 different populations. Symptoms have been evaluated on 420 of these. The remaining 150 accessions were inoculated, but complete evaluation was not possible due to too few replicates (plants died during assessment or there were not enough shoots per plant), and the result were not conclusive. Inoculation of these accessions will have to be repeated.

Evaluation of clones showed a normal distribution in their resistance to Witches' Broom disease (Figure 1.). This indicates that the chances of finding resistant and susceptible germplasm in the ICG,T are similar.

Figure 1. Distribution of resistance to Witches' Broom infection for 420 clones from the ICG,T.



Spray/agar-droplet experiments

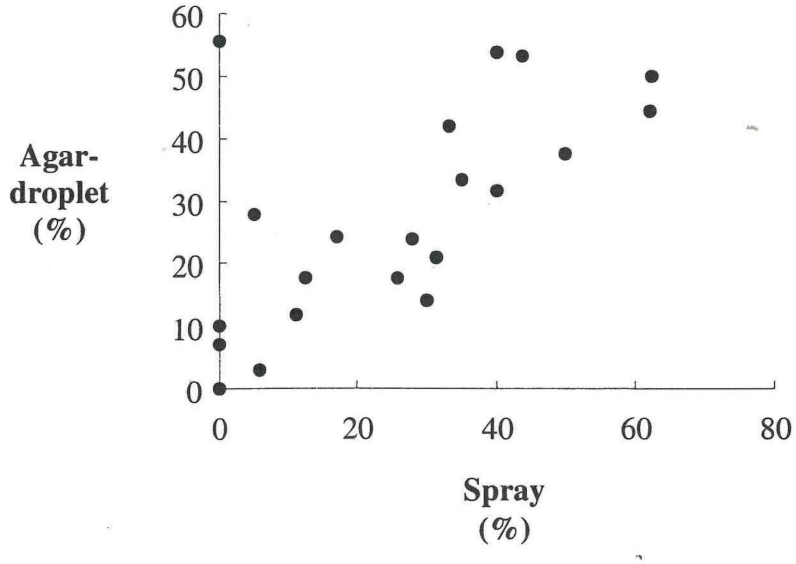
Results from spray and agar-droplet experiments proved to be very interesting (Table 1). Generally accessions seemed to show similar trends in percentage infection (all symptoms produced from total number of shoots inoculated per clone, calculated as a percentage), using

both techniques (Figure 1). In particular, with the exception of two accessions (IMC 2 and JA 5/25 [POU]), those with zero or very low percentage symptoms gave low infection rates for both techniques.

Table 1. Comparison of results from same plant inoculation using both spray and agar-droplet inoculation methods.

Clone	Infection (%)	
	Spray	Agar
B 5/3 [POU]	62.1	44.4
B 5/7 [POU]	33.3	41.9
CL 19/10	43.8	53.3
CLM 43	25.9	17.6
ICS 10	27.8	23.8
ICS 29	50.0	37.5
ICS 40	5.9	3.1
ICS 46	0.0	0.0
ICS 53	0.0	7.1
ICS 62	0.0	0.0
ICS 70	30.0	14.3
ICS 75	62.5	50.0
IMC 2	0.0	55.6
IMC 20	0.0	10.0
JA 5/25 [POU]	5.1	27.8
JA 5/34 POU]	31.3	21.1
M 33 [ICT]	40.0	53.9
NA 534	11.1	11.8
PA 136 [PER]	40.0	31.6
PA 150 [PER]	17.0	24.1
PA 187 [PER]	12.5	17.6
UF 12	35.1	33.3

Figure 2. Trends in percentage infection using two inoculation techniques.



Based on this evidence, we aim to continue to use the spray method for mass screening and the agar-droplet method for confirmation of the interesting accessions selected by the spray method.

Confirmation of resistance

For confirmation with agar-droplet inoculations, 27 accessions have already been propagated by micro-grafting from a potential list of 90 (Table 2). Apart from the selection of promising types, the current project has also been able to identify those accessions that are definitely susceptible to the disease. Also, once a final list of selected accessions is obtained, cross checks with data on infection in the field as well as performance with regard to other diseases, such as Black Pod, and with other characteristics such as Pod Index and flavour will be made. This will form the basis of a list of accessions from the ICG,T that will be a guide for selection of parents for germplasm enhancement and breeding work.

Table 2. Populations with promising clones.

Population	No. of clones	Population	No. of clones
AM [POU]	4	LV [POU]	2
B [POU]	10	MATINA	1
CL	2	MO	1
CRU	3	MOQ	3
CRUZ	1	NA	4
CBO [VEN]	1	POUND [POU]	2
DOM	1	PA [PER]	11
EET [ECU]	2	SCA	3
GU	5	SJ [POU]	3
ICS	8	SLA	1
IMC	10	SLC	1
LCT EEN	2	SPA [COL]	1
LP [POU]	6	UF	2

TSH screening

Broom data collected from 23 varieties were analysed by ANOVA using the general linear model (GLM), Minitab release 12.2 (Minitab Inc.). Out of the four responses analysed (broom-base diameter, broom weight, broom length and time to symptom), only broom-base diameter was significantly different among varieties at $P \leq 0.01$ (Table 3). The other three measurements were not significant.

Table 3. Analysis of variance for broom-base diameter × clone.

Source	DF	MS	F-ratio
Clone	29	9.619	2.91**
Error	57	3.303	
Total	86		

S.E. 1.0493 **significant at $P<0.01$

Many of the TSH varieties tested were non-commercial varieties, and the results indicate similar numbers resistant and susceptible varieties.

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